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(54) Title: METHOD FOR DETECTING DNA POLYMERISATION

(57) Abstract: A method for determining the extent of a processive nucleic acid polymerase reaction producing pyrophosphate can be conducted, in the presence of all components necessary for the progression of nucleic acid synthesis, wherein the components comprise a substrate for the nucleic acid polymerase which is either dATP or a dATP analogue. The method includes a pyrophosphate assay comprising the steps of conversion of the pyrophosphate to ATP and detection of light produced by the bioluminescence reaction of a luciferase with ATP, wherein one or both of the following apply: a) the luciferase reacts with ATP and the substrate, such that the spectral overlap is reduced relative to the spectral overlap between the outputs of reaction between wild-type *Photinus pyralis* luciferase with ATP and dATP respectively; and b) the luciferase reacts with the substrate to give a reduced bioluminescence relative to that produced by the reaction of wild-type *Photinus pyralis* luciferase with dATP.

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 02/00648

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, EMBASE, MEDLINE, BIOSIS, CHEM ABS Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 28440 A (DZIEGLEWSKA HANNA EVA ;PYROSEQUENCING AB (SE); NYREN PAAL (SE)) 2 July 1998 (1998-07-02) cited in the application page 3, paragraph 3 page 7, paragraph 3 -page 12, paragraph 1 page 16, paragraph 3 page 25, paragraph 2 page 28, paragraph 2 -page 29, paragraph 3 claims 1,6,9,16,17 --- -/--	1,3,4, 7-17

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*G\* document member of the same patent family

Date of the actual completion of the international search

17 February 2003

Date of mailing of the international search report

24/02/2003

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## INTERNATIONAL SEARCH REPORT

International application No

PCT/GB 02/00648

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>NYGREN M ET AL: "QUANTIFICATION OF HIV-1 USING MULTIPLE QUANTITATIVE POLYMERASE CHAIN REACTION STANDARDS AND BIOLUMINOMETRIC DETECTION" ANALYTICAL BIOCHEMISTRY, ORLANDO, FL, US, vol. 288, no. 1, 1 January 2001 (2001-01-01), pages 28-38, XP000977141 ISSN: 0003-2697 page 30, right-hand column, paragraph 2</p> <p>---</p>	1,3,4, 7-17
X	<p>WO 92 16654 A (UNIV REIMS CHAMPAGNE ARDENNE) 1 October 1992 (1992-10-01) cited in the application page 5, paragraph 6 -page 6, paragraph 1 page 13, paragraph 3 -page 18, paragraph 1 page 23, paragraph 1 -page 28, paragraph 1 claims 1,8,16,17</p> <p>---</p>	17
Y	<p>---</p>	1,3,4, 8-16
Y	<p>TABARY T ET AL: "HOMOGENEOUS PHASE PYROPHOSPHATE (PPI) MEASUREMENT (H3PIM). \A NON-RADIOACTIVE, QUANTITATIVE DETECTION SYSTEM FOR NUCLEIC ACID SPECIFIC HYBRIDIZATION METHODOLOGIES INCLUDING GENE AMPLIFICATION" JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol: 156, no. 1, 1992, pages 55-60, XP000320297 ISSN: 0022-1759 cited in the application page 56 -page 57</p> <p>---</p>	1,3,4, 8-16
A	<p>WHITE ET AL: "Improved thermostability of the north american firefly luciferase: saturation mutagenesis at position 354" BIOCHEMICAL JOURNAL, PORTLAND PRESS, LONDON, GB, vol. 319, 1996, pages 343-350, XP002097112 ISSN: 0264-6021 abstract page 347</p> <p>-----</p>	10

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/GB 02/00648

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1, 3, 4, 7-16 (partly) and 2, 5, 6 (completely)  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1, 3, 4, 7-16 (partly) and 2, 5, 6 (completely)

1. Present claims 1, 5 and 6 relate to a method/product defined by reference to a desirable characteristic or property, namely
- that the luciferase reacts with ATP and the substrate, such that the spectral overlap is reduced relative to the spectral overlap relative to... (claim 1 a))
  - that the luciferase reacts with the substrate to give a reduced bioluminescence relative to... (claim 1 b))
  - that the luciferase is a variant or mutant of a coleopteran luciferase (claim 5)
  - that the luciferase is a variant or mutant of *Photinus pyralis* luciferase (claim 6).

The claims 1-16 cover all methods/products having this characteristic or property, whereas the application provides support within the meaning of Art. 6 PCT and disclosure within the meaning of Art. 5 PCT for only a very limited number of such methods as far as claims 1, 3, 4 and 7-16 are concerned and none as far as claims 5 and 6 are concerned. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Art. 6 PCT). An attempt is made to define the method/products by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search with respect to claims 1, 3, 4, 7-16 has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the methods in which an dATP analogue is used which has a reduced bioluminescence when reacting with wild-type *Photinus pyralis* luciferase relative to that produced by the reaction of said luciferase with dATP. Since claim 2 refers to dATP being the substrate no search has been carried out on the subject-matter of said claim. Furthermore, the application does not disclose any variants or mutants of luciferases no search has been carried out with respect to claims 5 and 6.

2. Also claim 16 relates to a product defined by reference to a desirable characteristic or property, namely the stabilising effect of the factor. Again, although covering all products having this property, the application does not support (Art. 6 PCT) or disclose (Art. 5 PCT) any such stabilising factors. A further lack of clarity arises as said factors are considered to be defined by reference to a result to be achieved (Art. 6 PCT). Consequently, no search of claim 16 has been carried out with respect to said stabilising factor.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a

**FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210**

preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 02/00648

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9828440	A	02-07-1998	AU 5331198 A	17-07-1998
			DE 69713386 D1	18-07-2002
			DE 69713386 T2	30-01-2003
			EP 0946752 A1	06-10-1999
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			JP 2001506864 T	29-05-2001
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WO 9216654	A	01-10-1992	FR 2674254 A1	25-09-1992
			AU 1646092 A	21-10-1992
			WO 9216654 A1	01-10-1992
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